

BRIEF REPORT

The Putative Atypical Antipsychotic Drug Amperozide Preferentially Increases *c-fos* Expression in Rat Medial Prefrontal Cortex and Lateral Septum

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The effects of acute, systemic administration of the putative atypical antipsychotic drug amperozide on c-fos expression in the rat forebrain were studied by means of Fos immunohistochemistry. Amperozide significantly increased the number of Fos-immunoreactive nuclei in the medial prefrontal cortex and the lateral septum but not in the nucleus accumbens (shell or core), the striatum, or the amygdala. With the exception of the nucleus accumbens-shell, where amperozide failed to produce statistically significant increases, the regional distribution of Fos

immunoreactivity following amperozide was similar to that induced by atypical, but not by typical, antipsychotic drugs. In addition, after amperozide the number of Fos-positive nuclei was higher in the nucleus accumbens than in the dorsolateral striatum, a characteristic that is common to all known atypical antipsychotic agents.

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Amperozide is a new, putative atypical antipsychotic drug characterized by a high affinity for serotonin (5-HT)₂ receptors, and relatively low to moderate affinity for dopamine (DA) receptors (D₁ or D₂), α -adrenoceptors and other serotonergic receptors (Meltzer et al. 1989, 1992; Roth et al. 1992; Svartengren and Celander 1994; Svartengren and Simonsson 1990). The results of behavioral, biochemical, and electrophysiological stud-

ies suggest that amperozide exhibits a limbic mode of action (Grenhoff et al. 1990; Gustafsson and Christensson 1990; Pettersson et al. 1990). Recently, Pehek et al. (1993) and Nomikos et al. (1994) using *in vivo* microdialysis, and Marcus et al. (1996) using *in vivo* voltammetry, showed that like clozapine, amperozide increases DA release in the medial prefrontal cortex (mPFC) to a greater extent than in the nucleus accumbens (NAcc) or the striatum, and that it selectively enhances DA release in the shell subdivision of the NAcc, which is largely associated with limbic functions, but not in the motorically-related core subdivision of the NAcc. In addition, open clinical trials have suggested that amperozide may have antipsychotic actions, affecting both positive and negative symptoms, with a very low propensity to induce extrapyramidal side effects (Axelsson et al. 1991; Björk et al. 1992).

Immediate early genes, such as *c-fos*, have proven to be useful markers of changes in neuronal activity, and

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recently Fos immunohistochemistry has been used to examine the effects of antipsychotic drugs in the brain (Deutch et al. 1992; Dragunow et al. 1990; Guo et al. 1995; MacGibbon et al. 1994; Miller et al. 1990; Nguyen et al. 1992; Robertson and Fibiger 1992; Robertson et al. 1994). These studies have shown that typical and atypical antipsychotic drugs differentially affect *c-fos* expression in various regions of the brain. Thus, haloperidol induces *c-fos*, expression in the NAcc, the lateral septum and the striatum, whereas clozapine increases *c-fos* expression in the NAcc, the lateral septal nucleus, and the mPFC. In fact, based on calculations of the difference between the number of neurons displaying Fos-like immunoreactivity (FLI) in the NAcc and that in the dorsolateral striatum in response to several antipsychotic drugs, an index of atypicality has been proposed that is positive for atypical antipsychotics and negative for classical neuroleptics (Robertson et al. 1994). It is conceivable, therefore, that the regional specificity of antipsychotic-mediated immediately early gene expression may reflect different patterns of neuronal responses and consequently differences in the clinical profile between the typical and atypical antipsychotic drugs.

The purpose of the present study was to study the effects of acute, systemic administration of amperozide on the number and anatomical distribution of Fos-positive neurons in the basal ganglia and various limbic regions, particularly in the mPFC, the NAcc (shell and core), the striatum, the lateral septum, and the amygdala.

MATERIALS AND METHODS

Male Wistar rats weighing 300–400 g were used. All rats were housed individually for at least one week before the experiment and were handled for 5–10 minutes daily the last two days. Amperozide hydrochloride (Pharmacia Leo Therapeutics AB) was dissolved in 0.9% NaCl (saline) and injected subcutaneously (s.c.) in doses of 5.0 or 10.0 mg/kg (11.4 or 22.8 $\mu\text{mol}/\text{kg}$) in the neck; control animals were injected with saline (1.0 ml/kg). Two hours after saline or amperozide injection, all animals were deeply anesthetized with pentobarbital (100 mg/kg or 0.40 mmol/kg, intraperitoneally) and perfused transcardially with saline (200 ml) followed by 4% paraformaldehyde (300 ml) in phosphate-buffered (0.1 M) saline (PBS). Each brain was removed immediately after perfusion and placed in fixative for at least 12 hours.

Fos immunohistochemistry was performed as described by Robertson and Fibiger (1992) and Chergui et al. (1996). After the postfixative period, 30 μm coronal sections were cut from each brain using a vibratome. Sections were washed three times with 0.02 M PBS and then incubated in PBS containing 0.3% hydrogen peroxide. Sections were then washed three times in PBS containing 0.3% Triton-X, 0.02% sodium azide and Fos pri-

mary antibody (sheep polyclonal antibody, Cambridge Research Biochemicals OA-11-823) diluted 1:2000 for 48 hours. The sections were then washed three times with PBS and incubated with a biotinylated rabbit anti-sheep secondary antibody (Vector Laboratories) diluted 1:500 for 1 hour. The sections were washed three times with PBS and incubated for 1 hour with PBS containing 0.3% Triton-X and 0.5% avidin-biotinylated horseradish peroxidase complex (Vector Laboratories). After three washes in PBS the sections were rinsed in 0.1 M acetate buffer (pH 6.0). The reaction was visualized with the glucose oxidase-3,3'-diaminobenzidine-nickel method. The reaction was terminated by washing in PBS, and the sections were mounted on chrome-alum coated slides, dehydrated and prepared for microscopic observation.

The number of Fos-like-immunoreactive nuclei was counted under microscopic control within a grid placed over the mPFC, the NAcc, the medial and lateral striatum, the lateral septum, and the basolateral amygdala as illustrated in previous studies (Robertson and Fibiger 1992—Fig. 1; Brown et al. 1992—Figure 1). Specifically, the anterior-posterior coordinates (measured from bregma according to Paxinos and Watson 1986) of sections in which Fos-positive nuclei were counted were +2.7, +1.2, +1.0, +0.7 and –2.8 mm for the mPFC, the NAcc, the striatum, the septum, and the amygdala, respectively. The size of the grid was 500 \times 500 μm and labelled cells were counted at $\times 250$ magnification. In addition, Fos-positive cells were counted in the shell and core subdivisions of the NAcc as shown in the study by Chergui et al. (1996—Figure 2) and by Deutch et al. (1992—Figure 1). Although no marker such as calcium-binding protein was used to distinguish between these two parts of the NAcc, caution was taken to avoid overlap of the sampled areas for each structure by placing the grid (300 \times 300 μm) close to the anterior commissure for the core and between core and septum for the shell. For each brain, in both hemispheres and each structure, cells were counted by two observers in three slices and the mean value of 12 measurements was calculated.

A one-way analysis of variance (ANOVA) was performed on the cell count data for the two doses of amperozide and vehicle from each brain region. If the ANOVA was significant, multiple comparisons were performed using the Newman-Keuls test.

RESULTS

Both doses of amperozide significantly increased the number of Fos-positive nuclei in the mPFC ($p = 0.011$ and $p = 0.017$ for the 5.0 and 10.0 mg/kg dose, respectively) and the lateral septum ($p = 0.011$ for either the 5.0 or the 10.0 mg/kg dose; Figures 1 and 2). In the mPFC, the amperozide-induced Fos-positive cells were evenly distributed among the cortical layers, although rela-

tively fewer cells were found in the superficial compared to the deep layers. Amperozide tended to produce dose-related increases in FLI in the NAcc (shell), although this did not reach statistical significance. Similarly, amperozide did not significantly influence FLI in

the NAcc (core), the striatum (dorsolateral or medial), or the amygdala (Figure 2). The number of Fos-positive nuclei was also not significantly affected by amperozide when a larger part of the NAcc was considered (data not shown), as in the study by Robertson and Fibiger (1992).

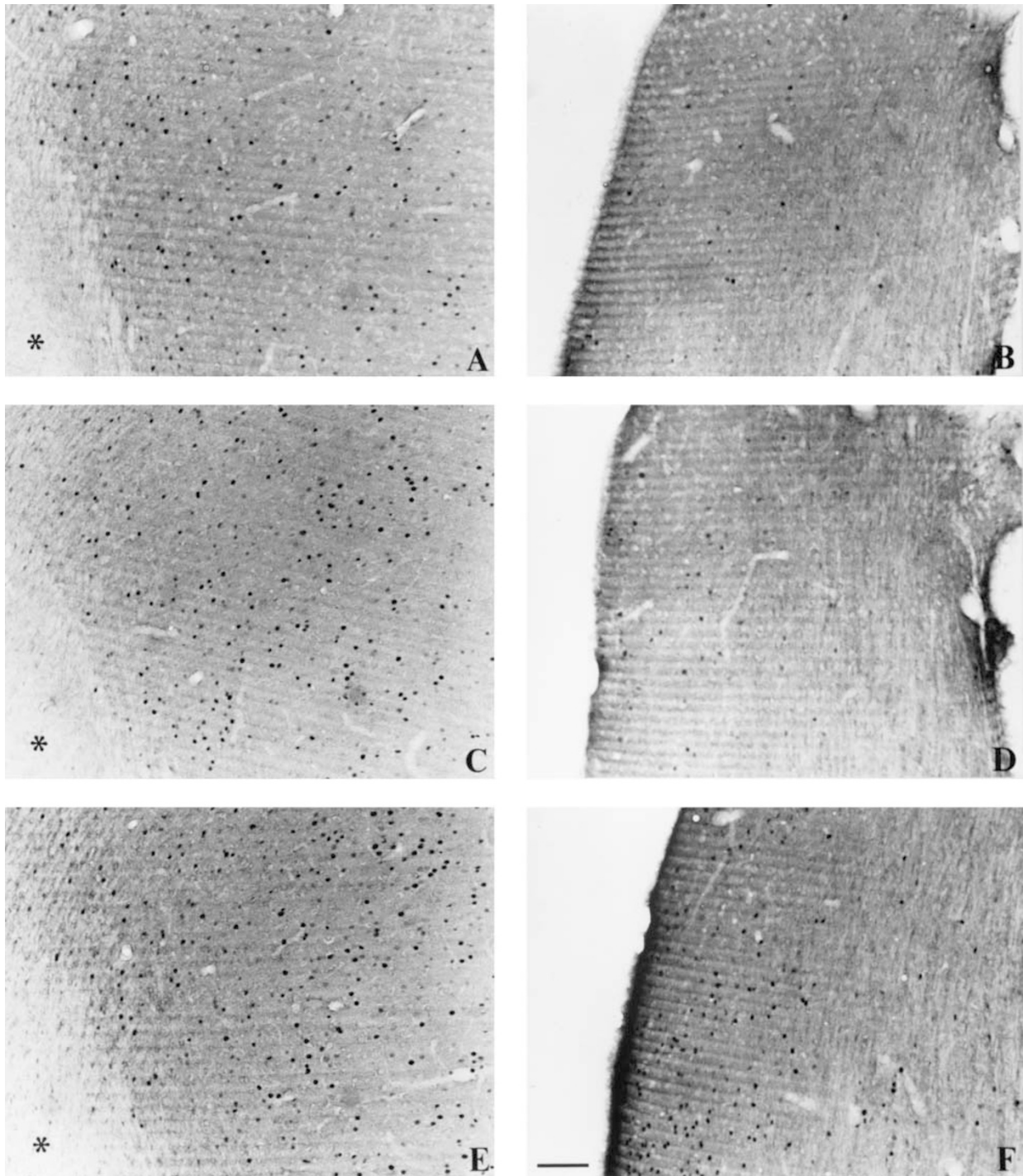


Figure 1. Low-magnification ($\times 100$) photomicrographs illustrating Fos-like immunoreactivity in the medial prefrontal cortex (A,C,E; stars indicate the forceps minor, corpus callosum) or the lateral septum (B,D,F) of the rat in response to saline (A,B), the 5.0 (C,D), or the 10.0 (E,F) mg/kg dose of amperozide. Scale bar = 100 μ m.

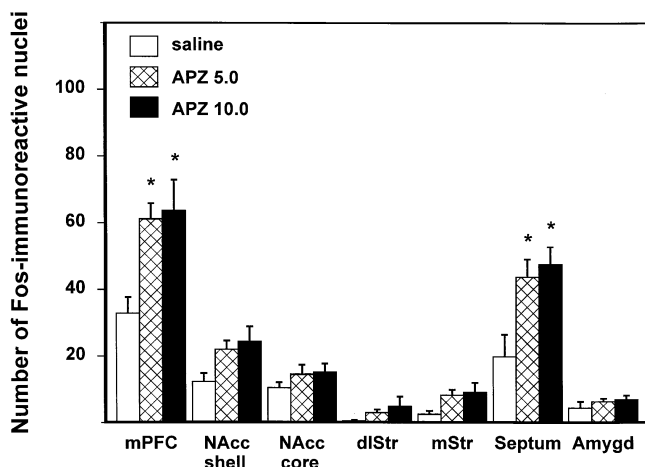


Figure 2. Effect of saline ($N = 5$) or amperozide (APZ; doses in mg/kg; $N = 6$ for each dose) on the number of Fos-like immunoreactive cells in the medial prefrontal cortex (mPFC), the shell or the core subdivision of the nucleus accumbens (NAcc), the dorsomedial striatum (dlStr), the medial striatum (mStr), the lateral septum and the basolateral amygdala (Amygd) of the rat. Bars indicate the mean (\pm SEM) number of Fos-like immunoreactive nuclei. * $p < 0.05$ compared to saline.

However, the calculated atypical index, i.e., the number of Fos-positive neurons in the NAcc minus the number in the dorsolateral striatum after correction for the injection procedure (Robertson et al. 1994), was positive for both the 5.0 (+9.5) and the 10.0 (+15.5) mg/kg dose of amperozide; statistical analysis revealed that the calculated atypical index in response to the high dose of amperozide was significantly ($p = 0.036$) different from that produced by the vehicle injection.

DISCUSSION

Like clozapine, amperozide significantly increased the number of Fos-immunoreactive nuclei in the mPFC and the lateral septum but not in the dorsolateral striatum. On the other hand, unlike clozapine, amperozide did not significantly influence FLI in the medial striatum or the NAcc. However, the index of atypicality calculated as the difference between the number of neurons displaying FLI in the NAcc and the dorsolateral striatum was positive for both doses, indicating that by this criterion amperozide can be classified as an atypical antipsychotic drug.

Based on *in vivo* receptor binding data (Meltzer et al. 1992; Svartengren and Celander 1994), the occupancy of 5-HT₂ receptors with the doses of amperozide used in the present study can be estimated to be high (>75%), whereas the D₂ receptor occupancy is probably low (<25%). Thus, the amperozide-induced *c-fos* expression

in the mPFC and the lateral septum may be related to its potent antagonist actions at 5-HT₂ receptors. However, previous studies have shown that ritanserin, a potent 5-HT₂ receptor antagonist, fails to induce *c-fos* expression in the relevant brain regions (Guo et al. 1995; Wan et al. 1995). These results suggest that antagonist actions of amperozide at 5-HT₂ receptors do not contribute to its effects on *c-fos* expression. Moreover, in a study by Guo et al. (1995), combined administration of ritanserin with haloperidol failed to mimic the pattern of *c-fos* expression produced by clozapine in the forebrain and particularly in the mPFC, although a similar combination of ritanserin with a low dose of raclopride, a selective D₂ receptor antagonist, results in a higher DA release in this brain region than raclopride alone (Andersson et al. 1995). In this regard, it is striking that the regional distribution of FLI produced by amperozide and clozapine, is similar to the pattern of their DA releasing effect in the forebrain, i.e., a more pronounced action in the mPFC than in subcortical dopaminergic regions (Moghaddam and Bunney 1990; Pehk et al. 1993; Nomikos et al. 1994; Robertson and Fibiger 1992).

Both doses of amperozide significantly increased *c-fos* expression in the lateral septum. The fact that almost all the clinically effective antipsychotic drugs, both typical and atypical, increase the number of Fos-immunoreactive neurons in the lateral septal nucleus (MacGibbon et al. 1994; Robertson and Fibiger 1992; Robertson et al. 1994; Wan et al. 1995) suggests that this part of the limbic system may be a significant part of the circuit mediating antipsychotic drug action. On the other hand, in contrast to all other antipsychotic agents tested to date (Deutch et al. 1992; Robertson et al. 1994; Robertson and Fibiger 1996), amperozide failed to significantly increase *c-fos* expression in the shell part of the NAcc. In this aspect, amperozide shows a unique profile with the implication that its putative antipsychotic action may be associated with actions in the brain that do not involve the shell of the NAcc. If future clinical studies indicate that amperozide is indeed a clinically effective antipsychotic, then it will be the first such agent that fails to significantly increase *c-fos* expression in the NAcc. This would have important implications regarding the brain regions that play a role in the therapeutic effects of antipsychotic drugs.

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